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Title of Invention

DIAGNOSTIC METHODS AND GENE THERAPY USING  
REAGENTS DERIVED FROM THE HUMAN METASTASIS SUPPRESSOR GENE  
KAI1.

Field of Invention

This invention is in the field of cancer  
diagnostics and therapeutics. In particular, this  
invention relates to detection of alterations of wild-type  
KAI1 gene sequence, KAI1 mRNA and KAI1 protein useful in  
determining the presence of malignant cancer in a subject  
or a genetic predisposition to malignancy in a subject.  
The invention further relates to the use of gene therapy  
to restore the wild-type KAI1 gene product.

Background of Invention

It has been widely accepted that carcinogenesis  
is a multistep process involving genetic and epigenetic  
changes that dysregulate molecular control of cell  
proliferation and differentiation. The genetic changes  
can include activation of proto-oncogenes and/or the  
inactivation of tumor suppressor genes that can initiate  
tumorigenesis as well as lead to the progression of  
tumors. For example, the tumor suppressor gene p53 may be  
involved in late stages of colorectal carcinomas (Baker,  
S.J. et al., (1989) Science, 244: 217-221) and a putative  
metastasis suppressor gene, nm23, was found down-regulated  
in metastatic tumors versus nonmetastatic tumors (Steeg,  
P.S. et al., (1988) J. Natl. Canc. Inst., 80:200-204). In  
addition, the activation of ras oncogene and the  
amplification of N-myc have been associated with  
progression of human tumors such as breast carcinomas  
(Liu, E. et al., (1988) Oncogene 3:323-327); and  
neuroblastomas (Brodeur, G.M. et al., (1984) Science,  
224:1121-1124; Schwab, M. et al., (1984) Proc. Natl. Acad.  
Sci. U.S.A., 81:4940-4944) but they are unlikely to be

universal determinants of tumor progression (Nicolson, G.L. Bio Essays, 13:337-342 (1991).

However despite these advances in understanding the genetic changes underlying carcinogenesis, metastasis, which is the main cause of death for most cancer patients (Rosenberg, S.A., Surgical Treatment of Metastasis Cancer (Lippincott, Philadelphia PA 1987)), remains one of the most important but least understood aspects of cancer (Liotta, L.A. et al. (1991) Cell, 64:327-336; Nicolson, G.L. (1991) BioEssays, 13:337-342 and Steeg, P.S. (1992) Curr. Opin. Oncol., 4:134-141). Accordingly, the isolation of metastasis tumor suppressor genes is of great importance for the diagnosis and therapy of cancers.

Cell fusion studies by Ramshaw et al. ((1983) Int. J. Cancer, 32:471-478) in which hybridization of non-metastatic and metastatic tumor cells produced cell hybrids which are tumorigenic but no longer metastatic demonstrated the existence of metastasis suppressor genes. More recently, Ichikawa et al. (1991) Cancer Res., 51:3788-3792) demonstrated that the metastatic ability of rat prostatic cancer cells was suppressed when fused to non-metastatic cancer cells and that the reexpression of metastasis was associated with the consistent loss of a normal rat chromosome. A subsequent study using micro-cell-mediated chromosome transfer further mapped a putative human metastasis suppressor gene to the 11p11.2-13 region of human chromosome 11. (Ichikawa et al. (1992) Cancer Res., 52:3486-3490) In this study, these researchers demonstrated that a hybrid retaining human chromosome 11cent-p13 showed a suppression of metastasis while hybrids retaining 11cent-p11.2 did not.

In sum, the data presented in the Ichikawa et al. papers suggested that a putative suppressor gene in the p11.2-13 region of human chromosome 11 may play a role in metastasis. However to date, no gene has been identified in this region which is a candidate metastasis

° suppressor gene. Thus, there is a need in the art to identify such gene(s) in this chromosome region and to determine if any such gene(s) is associated with metastasis.

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Summary of Invention

The present invention relates to methods for detecting alterations of the wild-type *KAI1* gene where detection of such alterations is useful in determining the presence of a malignant cancer in a subject or a genetic predisposition to malignancy in a subject. A first method  
10 for detecting alterations of the wild-type *KAI1* gene comprises analyzing the DNA of a subject for mutations of the *KAI1* gene. A second method for detecting alterations of the *KAI1* gene comprises analyzing the RNA of a subject  
15 for mutations and altered expression of the mRNA product of the *KAI1* gene.

The present invention therefore provides nucleic acid probes for detection of alterations of the wild-type *KAI1* gene.

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The present invention further provides a diagnostic kit containing purified and isolated nucleic acid sequences useful as PCR primers in analyzing RNA or DNA of a subject for alterations of the wild-type *KAI1* gene. These PCR primers can also be used to determine the  
25 nucleotide sequence of *KAI1* alleles.

A third method for detecting alterations of the wild-type *KAI1* gene comprises analyzing protein of a subject for alterations in the expression of *KAI1* protein.

The invention therefore relates to antibodies to  
30 the *KAI1* protein and to a diagnostic kit containing antibodies to *KAI1* protein useful for detecting alterations in *KAI1* protein expression in a subject.

The present invention further provides a method for supplying the wild-type *KAI1* gene to a cell having  
35 altered expression of the *KAI1* protein, the method

- ° comprising: introducing a wild-type *KAI1* gene into a cell having altered expression of *KAI1* protein such that the wild-type gene is expressed in the cell.

#### Description of Figures

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Figure 1 shows the results of a Northern blot of mRNA from normal tissue (human prostate) and from both metastatic (AT6.1, AT6.1-11-2 and AT6.1-11-3) and non-metastatic (AT6.1-11-1\*) tumor cells. 2 µg of poly A<sup>+</sup> RNA per sample was loaded in each lane and the blots were hybridized sequentially with *KAI1* cDNA and rat β-actin probes. The asterisk (\*) identifies the hybrid AT6.1-11-1 that contained the 11pcen-p13 region and was suppressed in metastatic ability.

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Figure 2 shows the results of Southern blot analysis of DNA isolated from human placenta, rodent cells (A9 and AT6.1) and human-rodent microcell hybrids (A9-11neo, AT6.1-11-1\*, AT6.1-11-2 and AT6.1-11-3). For each sample, 15 µg of DNA was digested with Hind III, separated on a 1.2% agarose gel and hybridized to a *KAI1* cDNA probe. As in Figure 1, the asterisk (\*) identifies the hybrid AT6.1-11-1 that contained the 11pcen-p13 region and was suppressed in metastatic ability.

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Figure 3 shows the nucleotide (upper line) and deduced amino acid (lower line) sequences of the *KAI1* cDNA where the abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr. The four putative transmembrane domains are noted by a dotted underline and the potential N-linked glycosylation sites are doubly underlined.

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Figure 4 shows the results of Northern blot analysis of 15 µg of total RNA isolated from human normal prostate tissue and from cell lines derived from human

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metastatic prostate cancers. The blot was hybridized sequentially with *KAI1* and human  $\beta$ -actin probes.

Figure 5 shows the results of Northern blot analysis of 2  $\mu$ g of poly A<sup>+</sup> RNA prepared from the various human tissues indicated at the top of Figure 5. The blot was hybridized sequentially with *KAI1* and human  $\beta$ -actin probes.

Figure 6 shows the results of a "zoo" blot of EcoRI-digested genomic DNA of the various species indicated at the top of Figure 6. The blot was hybridized with *KAI1* probe.

#### Detailed Description of Invention

The present invention relates to the cloning and characterization of a metastasis suppressor gene on human chromosome 11. The nucleotide and deduced amino acid sequences of this gene, designated *KAI1* herein, are shown in Figure 3. The nucleotide sequence shown in Figure 3 was cloned from a metastasis suppressed cell hybrid clone AT6.1-11-1\* and represents the wild-type *KAI1* sequence.

A search of the *KAI1* cDNA sequence in GenBank and EMBL databases revealed that the *KAI1* cDNA sequence is identical to three cDNA clones from human lymphocytes, designated C33, R2 and IA4 by different laboratories (Imai, T. et al. (1992) *J. Immunol.*, 149, 2879-2886 (1992); Fukudome, K. et al. (1992) *J. Virol.*, 66, 1394-1401 (1992); Gaugitsch, H. W., et al. (1991) *Eur. J. Immunol.*, 21, 377-383 (1991); Gil, M. L. et al. (1992) *J. Immunol.*, 148, 2826-33 (1992)). C33 is associated with the inhibition of virus-induced syncytium formation (Imai, T. et al. (1992); Fukudome, K. et al. (1992)); R2 is strongly up-regulated in mitogen-activated human T cells (Gaugitsch, H. W., et al. (1991)), and IA4 is highly expressed in several B lymphocyte lines (Gil, M. L. et al. (1992)). However, none of these three clones were suggested to function in metastasis and the function of

the protein encoded by these clones was not known prior to the present invention.

The present invention further relates to association of alterations of the wild-type *KAI1* gene with metastasis. Accordingly, the present invention relates to methods for detecting alterations of the wild-type *KAI1* gene in a subject where such methods can provide diagnostic and prognostic information.\* For example, since loss of expression of the *KAI1* gene has been observed in metastatic prostate tumors, these are tumors in which *KAI1* has a role in metastasis. Thus, detection of alterations of the wild-type *KAI1* gene in a subject may effect the course of treatment chosen by a clinician. In addition, since *KAI1* is expressed in all tissues tested including spleen, thymus, prostate, testes, ovary, small intestine, colon, blood leukocyte, heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas, alterations of the wild-type *KAI1* gene may contribute to metastasis in these tissues.

It is further understood by those of ordinary skill in the art that the methods of the present invention are applicable to any tumor in which alterations of wild-type *KAI1* occur. Moreover, the methods of detection disclosed in the present invention can be used prenatally to screen a fetus or presymptomatically to screen a subject at risk of having cancer based on his/her family history. For purposes of the present invention, subject means a mammal.

According to the diagnostic methods of the present invention, alterations of the wild-type *KAI1* gene are detected. "Alterations of the wild-type *KAI1* gene" as used throughout the specification and claims encompasses mutations of the wild-type *KAI1* gene where such mutations include deletions, inversions, insertions, transversions or point mutations of the wild-type *KAI1* gene. It is believed that many mutations found in tumor tissues will

° be those leading to decreased expression of *KAI1* protein. However, mutations leading to non-functional gene products can also lead to malignancy. It is further understood that point mutations can occur in regulatory regions (e.g. promoter) or can disrupt proper RNA processing thus  
5 leading to loss of expression of the *KAI1* gene products respectively.

"Alterations of the wild-type *KAI1* gene" as used throughout the specification and claims can also be detected on the basis of altered expression of the wild-  
10 type *KAI1*-specific mRNA and *KAI1* protein. The altered expression of these *KAI1* gene products may be detected as a loss or reduction in the levels of *KAI1* mRNA and protein relative to wild-type levels. Alternatively, the altered expression of *KAI1* protein can encompass a loss of  
15 function of the *KAI1* protein. Those of ordinary skill in the art would therefore understand that altered expression of the *KAI1* gene products may be caused by a variety of events, including but not limited to, mutations of the wild-type *KAI1* gene, changes in the posttranslational  
20 modification of the *KAI1* protein (e.g. glycosylation) or loss of a trans-acting factor necessary for transcription of the *KAI1* gene.

Provided with the *KAI1* cDNA and deduced amino acid sequences shown in Figure 3, design of particular  
25 probes useful in detecting alterations of the wild-type *KAI1* gene is well within the skill of the ordinary artisan.

In one embodiment of the invention, the method for detecting alterations of the *KAI1* gene comprises  
30 analyzing the DNA of a subject for mutations of the wild-type *KAI1* gene. For analysis of DNA, a biological specimen is obtained from the subject. Examples of biological specimens that can be obtained for use in the present methods include, but are not limited to, tissue  
35 biopsies, whole blood, lymphocytes and tumors. Means for

° enriching a tissue preparation for tumor cells are known in the art. For example, the tissue may be isolated from paraffin or cryostat sections. Cancer cells may also be separated from normal cells by flow cytometry and other techniques well known in the art. Alternatively, primary  
5 cell cultures can be established from tumor biopsies using methods known to those of ordinary skill in the art.

The DNA isolated from the biological specimen can be analyzed for mutations of the wild-type *KAI1* gene by a variety of methods including, but not limited to,  
10 Southern blotting after digestion with the appropriate restriction enzymes (restriction fragment length polymorphism, RFLP) (Botstein, D. (1980) Amer. J. Hum. Genet., 69:201-205, denaturing gradient electrophoresis technique (Myers, R.M., (1985) Nature, 313:495-498),  
15 oligonucleotide hybridization (Conner, R. et al., (1984) EMBO J., 3:13321-1326), RNase digestion of a duplex between a probe RNA and the target DNA (Winter, E. et al., (1985) Proc. Natl. Acad. Sci. U.S.A., 82:7575-7579),  
polymerase chain reaction (PCR) (Saiki, P.K. et al.,  
20 (1988) Science, 239:487-491; U.S. Patents 4,683,195 and 4,683,202), ligase chain reaction (LCR) (European Patent Application Nos. 0,320,308 and 0,439,182), and PCR-single stranded conformation analysis (PCR-SSCP) (Orita, M. et al. (1989) Genomics, 5:874-879; Dean, M. et al. (1990)  
25 Cell, 61:863-871).

In one preferred embodiment, Southern blot analysis can be used to examine DNA isolated from a subject for gross rearrangement of the *KAI1* gene. The DNA to be analyzed via Southern analysis is digested with one  
30 or more restriction enzymes. Following restriction digestion, resultant DNA fragments are separated by gel electrophoresis and the fragments are detected by hybridization with a labelled nucleic acid probe (Southern, E.M. (1975) J. Mol. Biol., 98:503-517).

35 The nucleic acid sequence used as a probe in



0 Southern analysis can be labeled in single-stranded or  
double-stranded form. Labelling of the nucleic acid  
sequence can be carried out by techniques known to one  
skilled in the art. Such labelling techniques can include  
5 radiolabels and enzymes (Sambrook, J. et al. (1989) in  
"Molecular Cloning, A Laboratory Manual", Cold Spring  
Harbor Press, Plainview, New York). In addition, there  
are known non-radioactive techniques for signal  
amplification including methods for attaching chemical  
10 moieties to pyrimidine and purine rings (Dale, R.N.K. et  
al. (1973) Proc. Natl. Acad. Sci., 70:2238-2242; Heck,  
R.F. 1968) S. Am. Chem. Soc., 90:5518-5523), methods which  
allow detection by chemiluminescence (Barton, S.K. et al.  
(1992) J. Am. Chem. Soc., 114:8736-8740) and methods  
15 utilizing biotinylated nucleic acid probes (Johnson, T. K.  
et al. (1983) Anal. Biochem., 133:126-131; Erickson, P.F.  
et al. (1982) J. of Immunology Methods, 51:241-249;  
Matthaei, F.S. et al. (1986) Anal. Biochem., 157:123-128)  
and methods which allow detection by fluorescence using  
commercially available products. Each of the nucleic acid  
20 sequences used as a probe in Southern analysis is derived  
from the wild-type *KAI1* gene. Preferred probes are  
derived from having the cDNA sequence shown in Figure 3.

Once the separated DNA fragments are hybridized  
to the labelled nucleic acid probes, the restriction  
25 digest pattern can be visualized by autoradiography and  
compared with the restriction digest pattern of the wild-  
type *KAI1* gene. The presence or absence of a restriction  
fragment length polymorphism (RFLP) in the restriction  
pattern of the subject's DNA relative to the wild-type  
30 restriction pattern indicates an alteration of the wild-  
type *KAI1* gene.

In another preferred embodiment, genomic DNA may  
be analyzed for mutations in the wild-type *KAI1* gene via  
PCR-SSCP. In this method, each of the pair of primers  
35 selected for use in PCR are designed to hybridize with

sequences in the wild-type *KAI1* gene to permit amplification and subsequent detection of mutations in the denatured amplification product via non-denaturing polyacrylamide gel electrophoresis. In one embodiment, primer pairs are derived from the *KAI1* cDNA sequence shown in Figure 3.

In another embodiment, primer pairs useful in the analysis of genomic DNA mutations<sup>®</sup> of the wild-type *KAI1* gene may be derived from intronic sequences which border the 5' and 3' ends of a given exon of the *KAI1* gene. Examples of primer pairs permitting specific amplification of specific exons of the *KAI1* gene include:

SEQ ID NO:1: AGAAGATCAAGTTGAAGAGG  
SEQ ID NO:2: GGGACCTCATTTCCTAGCTG  
SEQ ID NO:3: ATGAAACTGCTCTTGTCGG  
SEQ ID NO:4: TCAGCTCTTGGCTCCCCATT  
SEQ ID NO:5: TGGGCACGGGTTTCAGGAAAT  
SEQ ID NO:6: TGCAGAGAGCCCCAAATGCA  
SEQ ID NO:7: AGGGTGAGCCGTGAGCACAA  
SEQ ID NO:8: TGCTGAGAGTACCCAGATGC  
SEQ ID NO:9: GATGGCCACACCCACGCCC  
SEQ ID NO:10: TGCATGGAGAAGGTGCAGGC  
SEQ ID NO:11: CCTCTTGCCCACCCTGACTGA  
SEQ ID NO:12: TTCACACCATTCTCCTGCCT

where SEQ ID NOS:1 and 2 bound exon 3; SEQ ID NOS:3 and 4 bound exon 4; SEQ ID NOS: 5 and 6 bound exon 6; SEQ ID NOS:7 and 8 bound exon 7; SEQ ID NOS:9 and 10 bound exon 8; and SEQ ID NOS:11 and 12 bound exon 9.

Each primer of a pair is a single-stranded oligonucleotide of about 15 to about 50 base pairs in length which is complementary to a sequence at the 3' end of one of the strands of a double-stranded target sequence. Optimization of the amplification reaction to obtain sufficiently specific hybridization to the *KAI1* gene sequence is well within the skill in the art and is preferably achieved by adjusting the annealing

temperature. In yet another embodiment, RNA may be analyzed for mutations in the *KAI1* gene by RT-PCR-SSCP. In this method, single stranded cDNA is prepared from either total RNA or polyA<sup>+</sup>-enriched RNA using reverse transcriptase. In this method, each of the pairs of primers selected for use in PCR of the resultant single-stranded cDNA are designed to hybridize with sequences in the *KAI1* cDNA which are an appropriate distance apart (at least about 100-300 nucleotides) in the gene to permit amplification and subsequent detection of mutations in the denatured amplification product via non-denaturing polyacrylamide gel electrophoresis. Such primer pairs can be derived from the *KAI1* cDNA sequence set forth in Figure 3. Each pair comprises two such primers, complementary to sequences on each strand separated by generally about 100 to about 300 base pairs.

The primers of this invention can be synthesized using any of the known methods of oligonucleotide synthesis (e.g., the phosphodiester method of Agarwal et al. (1972) Agnew. Chem. Int. Ed. Engl., 11:451, the phosphotriester method of Hsiung et al. (1979). Nucleic Acids Res., 6:1371, or the automated diethylphosphoramidite method of Beuacage et al. (1981). Tetrahedron Letters, 22:1859-1862), or they can be isolated fragments of naturally occurring or cloned DNA. In addition, those skilled in the art would be aware that oligonucleotides can be synthesized by automated instruments sold by a variety of manufacturers or can be commercially custom ordered and prepared. In one embodiment, the primers can be derivatized to include a detectable label suitable for detecting and/or identifying the primer extension products (e.g., biotin, avidin, or radiolabelled dNTP's), or with a substance which aids in the isolation of the products of amplification (e.g. biotin or avidin).

The present invention therefore provides a

° diagnostic kit for detecting mutations of the *KAI1* gene. This diagnostic kit comprises purified and isolated nucleic acid sequences useful as hybridization probes or as PCR primers in analyzing DNA or RNA for alterations of the wild-type *KAI1* gene.

5 In an alternative embodiment, nucleic acid probes can be selected to hybridize to mutant alleles of the *KAI1* gene. These allele-specific probes are useful to detect similar mutations in other subjects on the basis of hybridization rather than mismatches. Where such nucleic  
10 acid probes are primer pairs which hybridize to mutations in the *KAI1* gene sequence, these primer pairs can be used to amplify specific mutant gene sequences present in a biological sample via PCR.

The amplification products of PCR can be  
15 detected either directly or indirectly. Direct detection of the amplification products is carried out via labelling of primer pairs. Labels suitable for labelling the primers of the present invention are known to one skilled in the art and include radioactive labels, biotin, avidin,  
20 enzymes and fluorescent molecules. The desired labels can be incorporated into the primers prior to performing the amplification reaction. A preferred labelling procedure utilizes radiolabelled ATP and T4 polynucleotide kinase (Sambrook, J. et al. (1989) in "Molecular Cloning, A  
25 Laboratory Manual", Cold Spring Harbor Press, Plainview, NY). Alternatively, the desired label can be incorporated into the primer extension products during the amplification reaction in the form of one or more labelled dNTPs. In the present invention, the labelled amplified  
30 PCR products can be analyzed for mutations of the *KAI1* gene via separating the PCR products by non-denaturing polyacrylamide gel electrophoresis, denaturing polyacrylamide gel electrophoresis (PCR-SSCP) or via direct sequencing of the PCR-products.

35 In yet another embodiment, unlabelled

amplification products can be analyzed for mutations in the *KAI1* disease gene via hybridization with nucleic acid probes radioactively labelled or, labelled with biotin, in Southern blots or dot blots. Nucleic acid probes useful in this embodiment are those described earlier for Southern analysis. In a further embodiment, detection of point mutations may be accomplished by molecular cloning of the allele present in the tumor tissue using the cDNA sequence set forth in Figure 3 and sequencing that allele using techniques well known in the art.

A second method for detecting alterations of the wild-type *KAI1* gene comprises analyzing the RNA of a subject for mutations and altered expression of *KAI1*-specific mRNA.

For the analysis of RNA by this method, RNA can be isolated from, for example, a tumor biopsy sample obtained from said subject where said tumors include, but are not limited to, prostate tumors.

The RNA to be analyzed can be isolated from blood or tumor biopsy samples as whole cell RNA or as poly(A)<sup>+</sup> RNA. Whole cell RNA can be isolated by methods known to those skilled in the art. Such methods include extraction of RNA by differential precipitation (Birnbiom, H.C. (1988) Nucleic Acids Res., 16:1487-1497), extraction of RNA by organic solvents (Chomczynski, P. et al. (1987) Anal. Biochem., 162:156-159) and extraction of RNA with strong denaturants (Chirgwin, J.M. et al. (1979) Biochemistry, 18:5294-5299). Poly(A)<sup>+</sup> RNA can be selected from whole cell RNA by affinity chromatography on oligo-d(T) columns (Aviv, H. et al. (1972) Proc. Natl. Acad. Sci., 69:1408-1412).

The methods for analyzing RNA for mutations and altered expression of *KAI1*-specific mRNA include Northern blotting (Alwine, J.C. et al. (1977) Proc. Natl. Acad. Sci., 74:5350-5354), dot and slot hybridization (Kafatos, F.C. et al. (1979) Nucleic Acids Res., 7:1541-1522),

° filter hybridization (Hollander, M.C. et al. (1990) Biotechniques; 9:174-179), S<sub>1</sub> analysis (Sharp, P.A. et al., (1980) Meth. Enzymol., 65:750-768), RNase protection (Sambrook, J. et al. (1989) in "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Press, Plainview, NY), reverse-transcription polymerase chain reaction (RT-PCR) (Watson, J.D. et al. (1992) in "Recombinant DNA" Second Edition, W.H. Freeman and Company, New York) and RT-PCR-SSCP.

10 Where expression of *KAI1* mRNA is measured, diminished *KAI1* mRNA expression is indicative of alteration of the wild-type *KAI1* gene. One preferred method for measuring alterations in the level of *KAI1*-specific mRNA expression is Northern blotting where the nucleic acid sequence used as a probe for detecting *KAI1*-specific mRNA expression is complementary to all or part of the *KAI1* cDNA sequence shown in Figure 3.

15 A second preferred method for measuring, alterations in the level of *KAI1*-specific mRNA expression is detection of *KAI1* mRNA expression via hybridization of a nucleic acid probe derived from *KAI1* cDNA sequence to RT-PCR products generated from RNA isolated from a biological sample.

20 A third method for detecting alterations of the wild-type *KAI1* gene comprises analyzing the protein of a subject for alteration of wild-type *KAI1* protein. In one embodiment, alteration of wild-type *KAI1* protein encompasses a loss or reduction in the level of expression of *KAI1* protein in a biological sample.

25 Examples of immunoassays useful in determining the level of expression of *KAI1* protein include, but are not limited to, immunoprecipitation, radioimmunoassay, Western blot assay, immunofluorescent assay, enzyme immunoassay, chemiluminescent assay, immunohistochemical assay and enzyme-linked immunosorbent assay (ELISA). In addition, the above immunoassays may be used in

combination such as immunoprecipitation followed by Western blot. The above methods are described in Principles and Practice of Immunoassay, Price and Newman, eds., Stochton Press, 1991. Such assays may be a direct, indirect, competitive or noncompetitive immunoassay as described in the art (Oelbrick, N. (1984) J. Clin. Chem. Clin. Biochem., 22:895-904). The protein to be analyzed by such methods may be obtained from biological samples such as tumor biopsies and the protein can be obtained as a crude lysate or it can be further purified by methods known to those of ordinary skill in the art including immunoaffinity chromatography using antibodies to the KAI1 protein (Sambrook, J. et al (1989) in "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Press, Plainview, NY). Alternatively, levels of KAI1 protein may be detected by immunohistochemistry of fixed or frozen tumor sections.

For detection of KAI1 protein by immunoassay, the present invention provides anti-KAI1 antibodies where such antibodies may be polyclonal or monoclonal. If polyclonal antibodies are desired, serum containing polyclonal antibodies to KAI1 protein can be used or the polyclonal antibodies can be purified from other antigens present in the serum by immunoaffinity chromatography. Alternatively, monoclonal antibodies directed against KAI1 can readily be produced by one of ordinary skilled in the art. Methods of producing monoclonal or polyclonal antibodies are known to one of ordinary skilled in the art (Goding, J.W. (1983) monoclonal antibodies: Principles and Practice, Plodermic Press, Inc., NY, NY, pp. 56-97; Hurn, B.A.L. et al. (1980) MetK. Enzymol., 70:104-141).

Suitable immunogens which may be used to produce the polyclonal or monoclonal antibodies of the present invention include cell lysate prepared from cells transfected with a recombinant KAI1 protein, partially or substantially purified recombinant or native KAI1 protein,

° or peptides derived from the *KAI1* amino acid sequence shown in Figure 3. When purification of the recombinant or native *KAI1* protein is desired, it can be accomplished by standard protein purification procedures known in the art which may include differential precipitation,  
5 molecular sieve chromatography, ion-exchange chromatography, isoelectric focusing, gel electrophoresis, affinity, and immunoaffinity chromatography and the like. In the case of immunoaffinity chromatography, the recombinant protein may be purified by passage through a  
10 column containing a resin which has bound thereto antibodies specific for the *KAI1* protein.

In a preferred embodiment, the immunogen is a recombinantly produced *KAI1* protein or fragments thereof. Production of recombinant *KAI1* protein or a fragment  
15 thereof may be directed by a natural or synthetic nucleic acid sequence inserted into a suitable expression vector. A preferred nucleic acid sequence is the *KAI1* cDNA sequence shown in Figure 3. In one embodiment, restriction digest fragments containing the full-length  
20 cDNA or fragments thereof containing a coding sequence for *KAI1* can be inserted into a suitable expression vector. By suitable expression vector is meant a vector that can function in eukaryotic or prokaryotic cells and is capable of carrying and expressing a nucleic acid sequence  
25 encoding the *KAI1* protein or a fragment thereof. Such vectors and their use in producing recombinant proteins are known to those of ordinary skill in the art (Sambrook, J. et al. (1989) in "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Press, Plainview, NY).

30 The immunogen of the present invention can be used in a suitable diluent such as saline or water, or in complete or incomplete adjuvants. Further, the immunogen may or may not be bound to a carrier to make the protein immunogenic. Examples of such carrier molecules include  
35 but are not limited to bovine serum albumin (BSA), keyhole



- ° limpet hemocyanin (KLH), tetanus toxoid, and the like. The immunogen can be administered by any route appropriate for antibody production such as intravenous, intraperitoneal, intramuscular, subcutaneous, and the like. The immunogen may be administered once or at  
5 periodic intervals until a significant titer of anti-KAII antibody is produced. The antibody may be detected in the serum using an immunoassay.

The antibodies or antigen binding fragments may also be produced by genetic engineering. The technology  
10 for expression of both heavy and light chain genes in E. coli is the subject of PCT patent applications; publication number WO 901443, WO 901443, and WO 9014424 and in Huse et al. (1989) Science, 246:1275-1281.

- Alternatively, anti-KAII antibodies can be  
15 induced by administering anti-idiotypic antibodies as immunogens. Conveniently, a purified anti-KAII antibody preparation prepared as described above is used to induce anti-idiotypic antibody in a host animal. The composition is administered to the host animal in a suitable diluent.  
20 Following administration, usually repeated administration, the host produces anti-idiotypic antibody. To eliminate an immunogenic response to the Fc region, antibodies produced by the same species as the host animal can be used or the Fc region of the administered antibodies can be removed.  
25 Following induction of anti-idiotypic antibody in the host animal, serum or plasma is removed to provide an antibody composition. The composition can be purified as described above for anti-KAII antibodies, or by affinity chromatography using anti-KAII antibodies bound to the  
30 affinity matrix.

- In an alternative embodiment, the antibodies of the present invention can be used in situ to detect KAII protein in cells or tissues. In one embodiment, the antibodies are used in direct or indirect  
35 immunofluorescence. In the direct method, anti-KAII

antibody labelled with a fluorescent reagent such as fluorescein isothiocyanate, rhodamine B isothiocyanate and the like is reacted directly with the KAI1 present in cells or tissues. In the indirect method, unlabelled anti-KAI1 antibody is reacted with the KAI1 protein present in cells or tissue. The unlabelled anti-KAI1 antibody is then reacted with a labelled second antibody. The second antibody can be labelled with a fluorescent tag as described above. The fluorescently labelled cells or tissues can then be detected using techniques known to one skilled in the art such as a fluorescence-activated cell sorter, light microscopy using a fluorescent light lamp and the like. Alternatively, KAI1 protein can be detected in situ via the use of radiolabelled anti-KAI1 antibody or via the use of an unlabelled anti-KAI1 antibody followed by a radiolabelled second antibody reactive to the anti-KAI1 antibody.

The antibodies of the present invention may also be used to immunoprecipitate the KAI1 protein from a mixture of proteins. The use of immunoprecipitation as a sensitive and specific technique to detect and quantitate target antigen in mixtures of proteins is well known to those of ordinary skill in the art (see Molecular Cloning, A Laboratory Manual, 2d Edition, Maniatis, T. et al. eds. (1989) Cold Spring Harbor Press).

The antibodies of the present invention may also be affixed to solid supports for use in the isolation of KAI1 protein by immunoaffinity chromatography. Techniques for immunoaffinity chromatography are known in the art (Harlow, E. and Lane, D. (1988) "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) including techniques for affixing antibodies to solid supports so that they retain their immunoselective activity; the techniques used may be those in which the antibodies are adsorbed to the support as well as those in which the antibodies are covalently

° linked to the support. Generally, the techniques are similar to those used in covalent linking of antigen to a solid support; however, spacer groups may be included in the bifunctional coupling agents so that the antigen binding site of the antibody remains accessible.

5 The above described antibodies and antigen binding fragments thereof may be supplied in a diagnostic kit useful for the detection of alterations in the expression of *KAI1* protein.

10 In a second embodiment, alteration of wild-type *KAI1* protein encompasses loss of function of the *KAI1* protein. The present method therefore includes assays useful in determining the functional status of the *KAI* protein. For example, since an association between processing of N-linked oligosaccharides and metastatic  
15 phenotype has been well-documented (Hakomori, S.-I. (1989) Advanc. Cancer Res., 52:257-331; Dennis, J. W., et al. (1987) Science, 236:582-585; Ishikawa, M. et al. (1988) Cancer Res., 48:665-670) it is believed that glycosylation of the *KAI1* protein is required for the protein to  
20 function as a metastasis suppressor. Thus, detection of a loss of function of *KAI1* protein as evidenced by an absence of glycosylation is indicative of the presence of metastatic cancer in a subject.

25 The present invention also relates to a gene therapy method in which an expression vector containing a nucleic acid sequence representing the wild-type *KAI1* gene is administered to a subject having a mutation of the *KAI1* gene. A nucleic acid sequence representing wild-type *KAI1* gene is that shown in Figure 3. Such nucleic acid  
30 sequence may be inserted into a suitable expression vector by methods known to those of ordinary skill in the art. Expression vectors suitable for producing high efficiency gene transfer in vivo include retroviral, adenoviral and vaccinia viral vectors.

35 Expression vectors containing a nucleic acid

sequence representing wild-type *KAI1* gene can be administered intravenously, intramuscularly, subcutaneously, intraperitoneally or orally. A preferred route of administration is intraperitoneally.

Any articles or patents referenced herein are incorporated by reference. The following examples are presented to illustrate various aspects of the invention but are in no way intended to limit the scope thereof.

### Examples

#### Materials and Methods

Cell lines: AT6.1 is a highly metastatic Dunning rat prostatic cancer cell line. AT6.1-11 clones are microcell hybrids that have a portion of human chromosomes 11 as the sole human genetic materials in AT6.1 cells. Microcell hybrid AT6.1-11-1\* contains a fragment of human chromosome 11, cen-p13, from the centromere to region p13, and was suppressed for metastatic ability. Microcell hybrids AT6.1-11-2 and -3 have smaller fragments of human chromosome 11 from the centromere to P11.2 and were not suppressed for metastatic ability. The characteristics and growth condition for these cell lines have been previously described in detail (Ichikawa et al, (1992) Cancer Res., 52:3486-3490). A9-11neo is a mouse A9 cell line containing human chromosome 11pter-q23 (Koi et al, (1989) Mol Carcinog., 2:12-21).

#### Isolation and sequencing of *KAI1* cDNA clone:

Poly (A)<sup>+</sup> RNA was isolated from exponentially growing AT6.1-11-1\* cells, using a FastTrack mRNA isolation kit (Invitrogen, San Diego, CA). Oligo (dT) was used to prime the first strand cDNA synthesis from 5 µg of poly (A)<sup>+</sup> RNA. Double-stranded cDNA was cloned into plasmid pSPORT 1 vector by procedures recommended by the vendor (GIBCO BRL, Grand Island, NY). Human Alu sequence primer Alu 559

° (Nelson, D.L. et al (189) Proc. Natl. Acad Sci U.S.A.  
86:6686-6690.) was used to amplify genomic DNA from  
suppressed hybrid AT6.1-11-1\* and the nonsuppressed clone  
AT6.1-11-2 by PCR. The multiple Alu-PCR fragments of  
AT6.1-11-1\* were cloned into a T-tailed vector pCR1000  
5 (Invitrogen, San Diego, CA). Individual clones  
corresponding to each fragment of Alu-PCR products were  
isolated after comparing the size of these Alu-PCR  
products to molecular weight markers in a agarose gel  
stained with ethidium bromide. Eleven fragments unique to  
10 AT6.1-11-1\* were labeled by random priming (GIBCO BRL,  
Bethesda, MD) and used to screen  $5 \times 10^4$  recombinants of  
the cDNA library under stringent wash conditions (65°C in  
0.1 x SSC + 0.1% SDS for 30 min.). Five independent clones  
were obtained and their inserts were sequenced using the  
15 Sequenase kit (US Biochemical, Cleveland, OH). DNA  
sequences were analyzed with the GCG package (version 7.3,  
1993, Madison, WI).

RNA analysis: Cytoplasmic RNA from AT6.1,  
AT6.1-11-1\*, -2 and -3 were prepared from exponentially  
20 growing cells, using FastTrack mRNA isolation kit  
(Invitrogen, San Diego, CA). Other poly (A)<sup>+</sup>RNA and human  
multiple tissue Northern blots were purchased from  
Clontech (Palo Alto, CA). 2 µg of poly (A)<sup>+</sup>RNA was  
denatured with formamide and fractionated on a 1.2%  
25 agarose gel in formaldehyde buffer. The RNA was then  
transferred onto nylon membrane, baked in an oven at 80°C  
for 90 min, and then hybridized with a labeled probe in  
QuickHyb hybridization solution (Stratagene, La Jolla, CA)  
at 68°C for 1.5 hours and washed at 68°C for 30 minutes in  
30 0.1% x SSC, 0.1% SDS and autoradiographed.

DNA analysis: 15 µg of genomic DNA was digested  
with BamHI and separated on a 1.2% agarose gel. Following  
denaturation and neutralization, the DNA in the gel was  
transferred onto nylon membrane. The "zoo" blot  
35 containing EcoRI-digested genomic DNA from human, rat,

mouse, dog, cow, rabbit, chicken and yeast (Zoo-blot) was purchased from Clontech (Palo Alto, CA). The Southern blots were further hybridized and washed under the same conditions as described above for the Northern blots.

5        PCR: All PCRs in this study were carried out in 50  $\mu$ l with 25 pmol of each primer, 10 mM Tris.HCl, 500 mM KCl, 1.5 mM  $MgCl_2$ , 0.01% gelatin, 250 mM of each dNTP and .25 units of Taq DNA polymerase (Perkin-Elmer, Norwalk, CT). The initial DNA denaturation was performed at 95°C for 5 min, followed by 35 cycles of 94°C denaturation for 1 min, 55°C annealing for 1 min and 72°C extension of 4 min, with a final extension of 72°C for 8 min.

10        Probes and Oligonucleotides sequences: The KAI1 probe (nucleotides 64-1094 of the KAI1 cDNA) used in Southern and Northern blot analyses was generated by PCR with primers shown as SEQ ID NO: 13 AGTCCTCCCTGCTGCTGTGTG and SEQ ID NO:14 TCAGTCAGGGTGGGCAAGAGG. Human and rat  $\beta$ -actin probes were PCR products generated by templates and primers purchased from Clontech (Palo Alto, CA). The primer sequences for human  $\beta$ -actin are shown as SEQ ID NO:15 GAGGAGCACCCCGTGCTGCTGA and SEQ ID NO:16 CTAGA AGCATTTGCGGTGGACGATGGAGGGGCC and the primer sequences for rat  $\beta$ -actin are shown as SEQ ID NO: 17 TTGTAACCAACTGGGACGATATGG and SEQ ID NO:18 GTCTTGATCTTCATGGTGCTAGG.

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#### Example 1

##### Cloning of the KAI1 Gene

To clone the gene on human chromosome 11 responsible for the metastasis suppression of AT6.1 prostatic cancer cells, genomic DNA fragments from the p11.2-13 region were isolated using human-specific Alu element-mediated PCR (Alu-PCR) (Nelson, D.L. et al Proc. Natl. Acad. Sci. U.S.A., 86:6686-6690) with DNAs from the metastasis suppressed microcell hybrid AT6.1-11-1\* and the non-suppressed hybrids AT6.1-11-2 and AT6.1-11-3. The

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Alu-PCR fragments found only in the AT6.1-11-1 DNA were then used as probes to screen a cDNA library prepared from the suppressed cell hybrid clone AT6.1-11-1\* that contains human chromosomal region 11cen-p13. Of five cDNA clones obtained, all were expressed in the suppressed hybrid but not in the nonsuppressed hybrids as detected by reverse transcription-polymerase chain reaction (RT-PCR) using primers derived from these cDNA sequences. Northern analysis of RNA isolated from human prostate and cell lines AT6.1, AT6.1-11-1\*, -2 and -3 revealed that two of the cDNA clones detected a 2.4 kb and 4.0 kb transcript respectively in human tissue and the suppressed AT6.1-11-1\* cells. The results of a Northern blot for one such clone, designated *KAI1* for Kang Ai (Chinese for anti-cancer), are shown in Figure 1 and clearly demonstrate that *KAI1* mRNA was abundant in the metastatic suppressed AT6.1-11-1\* cells but absent from the parental AT6.1 cells and the nonsuppressed hybrids. Therefore, the *KAI1* clone was analyzed further.

To confirm that the *KAI1* gene was isolated from the p11.2-13 region of human chromosome 11 involved in metastasis suppression, Southern blot analysis was conducted on 15  $\mu$ g of genomic DNA from human placenta, rodent cells (A9 and AT6.1) and human-rodent microcell hybrids (AT6.1-11-1\*, AT6.1-11-2 and AT6.1-11-3), digested with Hind III, separated on a 1.2% agarose gel and hybridized with *KAI1* probe. The results shown in Figure 2 demonstrate that only the cell hybrids that have the p11.2-13 region involved in metastasis suppression (AT6.1-11-1\*) have the pattern observed with normal human DNA when hybridized to *KAI1* probe. Fluorescence *in situ* hybridization of a *KAI1* probe to metaphase chromosomes further localized *KAI1* to the p11.2 region.

Example 2

Nucleotide And Deduced Amino Acid Sequences of  
the KAI1 cDNA

5 The nucleotide and deduced amino acid sequences  
of the KAI1 cDNA are shown in Figure 3. The KAI1 cDNA has  
a single open reading frame from nucleotide positions 166  
to 966, predicting a protein of 267 amino acids with a  
calculated molecular weight of 29,610 daltons. An Alu  
10 element was present in the 3'-untranslated region of the  
cDNA. The predicted protein had four hydrophobic and  
presumably transmembrane domains and one large  
extracellular hydrophilic domain with three potential  
N-glycosylation sites. As noted earlier, the KAI1 cDNA  
sequence is identical to three cDNA clones from human  
15 lymphocytes, C33, R2 and IA4.

Example 3

Determination That KAI1 Is A Metastasis Suppressor Gene

20 To investigate if KAI1 is the gene responsible  
for metastasis suppression in AT6.1-11-1\*, KAI1 cDNA was  
subcloned into a constitutive expression vector and  
transfected into parental AT6.1 cells as follows. In  
brief, KAI1 cDNA was cloned into pCMVneo, in which  
transcription is driven by the constitutive human  
25 cytomegalovirus promoter (Eliyahu, D. et al Proc Natl Acad  
Sci U.S.A., (1989) 86:8763-8767). The resultant plasmid  
pCMV-KAI1 was transfected into AT6.1 cells by calcium  
phosphate precipitate method and the vector alone was also  
transfected as a negative control. Individual  
30 transfectants were isolated in selection medium (RPMI-1640  
plus 10% fetal calf serum, 2units/ml pen-strep and 500  
ug/ml neomycin). Exponentially growing untransfected  
AT6.1, AT6.1-11-1\* and AT6.1-11-2 cells and exponentially  
growing vector (AT6.1VEC-1, AT6.1VEC-2 and AT6.1VEC-3) and  
35 KAI1 (AT6.1KAI-1, AT6.1KAI-2 and AT6.1KAI-3) transfectants



- ° were collected by scraping and cell clumps were broken up by gentle pipetting. The cell suspension was placed in a tube and allowed to stand at room temperature for 30 min. Cells from the supernatant suspension were collected, washed, and resuspended in cold PBS at  $10^6$  cells/ml.
- 5 Four-to-five-week-old male Ncr nu/nu nude mice (National Cancer Institute Animal Program, Bethesda, MD) were injected with 0.1 ml of the indicated<sup>6</sup> cell suspension ( $10^5$  cells) (the column designated "Clone" in Table 1) subcutaneously at sites on both the right and left
- 10 midlateral, about 1/4 of the distance from the base of the skull to the base of the tail. About 6 weeks after injection, the tumors were weighed and the lungs were inflated with Bouin's solution. Tumor foci on the surface of lungs were scored under a dissecting microscope.
- 15 Individual transfectants were analyzed for *KAI1* expression and for their ability to suppress lung metastases and the results of one experiment are shown in Table 1.

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TABLE 1

	Clones	<i>KAI1</i> <sup>*</sup> mRNA Level	Latency <sup>+</sup> (days)	Tumor Age (Days)	Tumor Weight(g) @ Excision	Number of Mice with Metastases	Mean Number <sup>‡</sup> Metastasis Per Mouse Mean (#mice)	P
5	AT6.1	0	4.3	27	2.58	19/19	58(32-135)	
	AT6.1-11-1*	10	3.7	37	2.79	6/7	7(0-9)	<0.005 <sup>‡</sup>
	AT6.1-11-1-2	0	4.2	37	2.78	6/6	26(20-40)	
10	AT6.1VEC-1	0	4.9	43	2.32	17/17	30(16-57)	
	AT6.1VEC-2	0	4.0	43	3.26	17/17	30(12-71)	
	AT6.1VEC-3	0	5.5	43	2.57	18/18	47(15-183)	
	AT6.1KAI-1	10	4.2	43	3.99	18/20	6(0-14)	<0.001 <sup>‡</sup>
15	AT6.1KAI-2	7	4.5	41	1.79	17/19	7(0-17)	<0.001 <sup>‡</sup>
	AT6.1KAI-3	1	4.5	43	2.56	18/19	23(0-36)	<0.02 <sup>‡</sup>

The data shown in this table are from a large, age-matched cohort of "side-by-side" nude mice, with cells inoculated at the same time.

\* *KAI1* expression was determined by Northern blot analysis. The *KAI1* signals on the Northern blot were scored by a densitometer. The value for AT6.1KAI-1 was standardized to 10 and the values for other clones were adjusted accordingly.

+ Latency is the time following injection for a palpable tumor to appear.

‡ The numbers in parentheses indicate the range of metastases in individual mice.

‡ Compared to the number of metastases with AT6.1-11-2 cells.

‡ Compared to the mean number of metastases with all of the three vector transfectants.

The results presented show that expression of *KAI1* resulted in a significant suppression of the number of lung metastases per mouse but did not affect the growth rate of the primary tumor. Further, whereas the parental AT6.1 cells yielded 58 metastasis per mouse when injected subcutaneously into nude mice, two transfectants with levels of *KAI1* mRNA expression similar to the high level of expression observed in AT6.1-11-1\* cells gave only 6 or 7 lung metastases per animal. In contrast, the three

vector control transfectants produced 30-47 lung metastases per mouse, which is on average 5.5 times the number of metastases observed with the 2 KAI1 transfectants with high KAI1 mRNA expression (AT6.1KAI-1 and AT6.1KAI-2). In addition, while the AT6.1KAI-3 clone which had low KAI1 expression produced 23 lung metastases, this was still significantly less than the mean number of lung metastases for control transfectants. Finally, Northern analysis showed that KAI1 expression was undetectable or very low in 28 lung metastases from KAI1 transfectants suggesting that selection for cells with absent or reduced KAI1 expression resulted in metastasis formation. These results indicate that the metastatic ability of AT6.1 cells is suppressed by KAI1 expression.

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Example 4.

KAI1 mRNA Expression In Cell Lines Derived  
From Metastatic Human Prostate Tumors

To determine whether KAI1 mRNA expression was reduced in human metastatic prostate tumors relative to expression in normal human prostate, 15 µg total RNA from human normal prostate tissue and from cell lines derived from metastatic prostate cancers (Kaighn, M.E. et al (1979) Invest. Urol.:17:16; Horoszewicz, J.J. et al. in Models for Prostate Cancer, G.P. Murphy. Ed. (Alan R. Liss, Inc., New York, 1980). pp 115-132; T. Iizumi. et al. (1987) J. Urol. 137:1304, D.D. Mickey et al., in Models for Prostate Cancer. G. P. Murphy. Ed. (Alan R. Liss, Inc. New York, 1980), pp. 67-84) were denatured with formamide, electrophoresed fractionated on a 1.2% agarose gel and hybridized sequentially to KAI1 and human β-actin probes. The results of this Northern blot analysis are shown in Figure 4 and clearly demonstrate that KAI1 expression was significantly reduced in the human cell lines derived from metastatic prostate tumors (PC-3, LNCaP, TSU-Pr1 and DU145) when compared to normal prostate

(prostate). In addition, while longer exposures (4 days at -80 C) of the autoradiogram shown in Figure 4 (overnite at -80 C) revealed expression of *KAI1* mRNA in all of the tumor cells, the level of expression was still much lower than in normal prostate.

5           To rule out the possibility that the metastasis suppression by *KAI1* was due to an indirect immune mechanism, two other experiments were performed. First, parental AT6.1 cells, cell hybrid clone AT6.1-11-1\*, or a *KAI1* transfectant (AT6.1 KAI-1) were inoculated into the  
10 leg of severe combined immune deficient (SCID) mice at  $5 \times 10^5$  cells/mouse. When tumors reached 3-5 cm<sup>3</sup>, the leg with tumor was surgically removed and animals were followed until 50 to 60 days post inoculation. Lung metastases for each mouse were analyzed as described for Table 1. For  
15 AT6.1, 9/9 mice had lung metastases with an average number of 83 per mouse. For AT6.1-11-1\*, 4/9 mice had lung metastases with an average number of 6 per mouse. For AT6.1KAI-1, 2/7 mice had lung metastases with an average number of 2 per mouse. These studies demonstrated that  
20 even in SCID mice, which are more immune compromised than nude mice, metastasis suppression was observed.

          Second, highly metastatic rat mammary cancer cells into which the *KAI1* gene was introduced via microcell-mediated chromosome transfer, retained their  
25 ability to metastasize (Rinker-Schaefer, C.W. et al. (1994) Cancer Res., 54:6249-6256) even though the hybrids expressed similar level of *KAI1* mRNA. Based upon these data, a more direct mechanism appears to be responsible for the metastasis suppression by *KAI1*. Consistent with  
30 this possibility, high *KAI1* expressing AT6.1-11-1\* hybrid cells have about 50% reduction in their invasive ability as compared to parental AT6.1 cells or nonsuppressed AT6.1-11-2 hybrid cells in Boyden chamber assay. In brief, Boyden chamber invasion assays were performed as  
35 described by J. Vukanovic et al. (1993) Cancer Res., 53:

1833), using matrigel coated filters and 5% fetal bovine serum as chemoattractant in the lower well. During the 12 hours of the assay,  $19 \pm 3$  parental AT6.1 cells per high power field invaded through the matrigel filters versus  $10 \pm 2$  for the metastasis suppressed AT6.1-11-1\* hybrid cells and  $18 \pm 2$  for the nonsuppressed AT6.1-11-2 hybrid cells.

#### Example 5

##### Expression of KAI1 Gene In Human Tissues

To evaluate the expression level of KAI1 gene in various human tissues, Northern analysis was performed on RNA isolated from multiple human tissues. In brief, a human multiple tissue Northern blot purchased from Clontech (Palo Alto, CA) was hybridized sequentially with KAI1 and human  $\beta$ -actin probes under conditions described in the Methods section. The results presented in Figure 5 show that the 2.4 kb KAI1 transcript was detected in all the human tissues tested, with high abundance in prostate, lung, liver, kidney, bone marrow and placenta; moderate abundance in mammary gland, pancreas, skeletal muscle and thymus; and low expression in brain, heart, ovary, stomach and uterus.

#### Example 6

##### Conservation Of The KAI1 Gene Across Species

To determine if the KAI1 gene is evolutionarily conserved across species, a zoo blot containing EcoRI-digested genomic DNA from various species was purchased from Clontech (Palo Alto, CA) and hybridized with KAI1 probe. The results presented in Figure 6 show that the evolutionary conservation of KAI1 coding sequence is high in human, monkey, dog and rabbit and moderate in cow, rat, mouse. The evolutionary conservation and wide tissue distribution for KAI1 suggest that the gene may have an essential biological function.

Example 7

Correlation Of Altered KAI1 Expression  
In Human Tissue Samples With Metastasis

Tumor biopsies of liver metastases from prostate cancer patients and liver biopsies from healthy patients are analyzed for KAI1 mRNA expression by Northern blotting. KAI1 mRNA expression is lost in the tumor samples indicating that the presence of liver metastases in the prostate cancer patients is correlated with altered KAI1 expression.

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